helix, or adjacent alpha helices, depend on enzyme transport activity, with the adjoining extracellular loop favoring binding of positively-charged ions. One interpretation of these data is that conformational changes involving the first transmembrane alpha helix affect the disposition of H_2O molecules in the membrane domain of the enzyme. These data, together with crystallographic data for other P-type ATPases, suggest that this region in the enzyme's transmembrane domain contains an extracellularly-facing cavity. We speculate that this cavity could contribute to a release pathway for H^+ during the ion transport cycle.

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Electrogenicity of Vacuolar H^+-ATPases at the Plasma Membrane of Osteoclasts

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The vacuolar-type H^+-ATPase (V-ATPase) is an electrogenic H^+ pump that is distributed widely in living organisms. Electrogenicity contributes to the proton motive force, but its precise evaluation is difficult mostly because V-ATPases are abundant at the intracellular membranes of acidic vesicles where fluxes of counter ions and H^+-leakage could not be fully controlled. In osteoclasts, bone-resorbing cells, V-ATPases are recruited to the plasma membrane (the ruffled membrane) by exocytotic fusion of lysozomes. The electogenicity of the plasma membrane V-ATPases was evaluated under the whole-cell current-clamp recordings in the absence of Na^+, K^+ and Cl^-, where H^+ was a major determinant of the membrane potentials. Under pH_o/pH_i 7.3/5.5 (ΔpH 1.8), the membrane potential varied greatly among cells, from −250 - −7 mV. Bafilomycin, a specific blocker for V-ATPases, depolarized cells by several to 220 mV. The depolarization was dependent on the amplitudes of the V-ATPase currents and eliminated by replacing intracellular ATP by ADP. The V-ATPase-mediated potential was reduced by decreasing ΔpH and disappeared at near ΔpH −2.5. As the whole-cell leak conductance was ~0.23 nS (the area resistance of the plasma membrane: 6 x 10^5 Ω cm^2), 10 pA of V-ATPase currents could yield the membrane potential of ~ 40 mV. Small ΔpH-dependent potential due to H^+ leakage currents remained in the presence of bafilomycin. Potentials generated by V-ATPases and H^+ leak are thus identified successfully. The ruffled membrane could provides a useful model for investigating H^+ fluxes across the membrane energized by V-ATPases.

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Kinetic Equivalence of pH Difference (ΔPh) and Electrical Potential ($\Delta\psi$) across Membrane in ATP Synthesis by Bacillus PS3 FOF1-ATP Synthase

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The ATP synthase is the key player of Mitchell's chemiosmotic theory, converting the energy of transmembrane proton flow into the high-energy bond between ADP and phosphate. The proton motive force that drives this reaction consists of two components, the pH difference (ΔpH) across the membrane and transmembrane electrical potential ($\Delta\psi$). The two are considered thermodynamically equivalent, but kinetic equivalence in the actual ATP synthesis is not warranted and previous experimental results vary. Here we show that, in the thermophilic Bacillus PS3 ATP synthase that lacks an inhibitory domain of the ε subunit, ΔpH imposed by acid-base transition and $\Delta\psi$ produced by valinomycin-mediated K+ diffusion potential contribute equally to the rate of ATP synthesis, within the experimental range examined (ΔpH −0.3 to 2.4, $\Delta\psi$ −30 to 140 mV, pH around the catalytic domain 8.0). Either ΔpH or $\Delta\psi$ alone can drive synthesis, even when the other slightly opposes.

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Interactions Between the γ Subunit and the ε Subunit Mediate Inhibition and Coupling in Escherichia Coli F1-ATPase

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The ε subunit of the F1 sector of the Escherichia coli ATP synthase is part of the rotor complex (γ-ε-c10). The ε subunit has been implicated in coupling, as its presence allows for the maintenance of the proper rate limiting transition state structure necessary for coupling of proton transport to ATP hydrolysis or synthesis (Peskova, Y.B. and Nakamoto, R.K., Biochemistry 39: 11830-11836, 2000). The ε subunit has also been shown to inhibit ATP hydrolysis. The two structural domains of the ε subunit have been thought to play different roles: the N-terminal β-sandwich domain plays a structural role in attaching the F1 complex to the membrane-bound FO and is sufficient for coupling between the two domains, while the C-terminal α-helical domain plays a role in the inhibition of hydrolysis activity. By characterizing steady state ATPase activity and isokinetic analysis of a series of mutant enzymes of F1 and FOF1, including point mutants, site-directed disulfide cross-linked mutants, and truncation mutants that disrupt or stabilize the interactions of the two ε subunit domains with the γ subunit, we show that two functions of the ε subunit are separable. The proper interaction of the N-terminal domain with γ is necessary not only for structural attachment but also to achieve the optimal transition state structure required for coupling, while the C-terminal domain interactions with γ and the $\alpha\beta$ hexamer modulate hydrolysis activity. Furthermore, we show that the conformation of the ε subunit C-terminal domain in the active state of the enzyme changes upon binding of F1 to FO, resulting in the observed increase in hydrolysis activity.

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The Phytopolyphenol Piceatannol Inhibits the Rate Limiting Step of Rotational Catalysis of the F1-ATPase

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Single molecule observations of the F1 sector of the E. coli ATP synthase were made in Vmax conditions with minimal load. The enzyme rotates through continuous cycles of catalytic dwells (pauses lasting ~0.2 ms) and 120° rotation steps (~0.6 ms in duration). We previously established that the rate limiting transition state step occurs during the catalytic dwell just prior to the initiation of the 120° rotation. Here we use the phytopolyphenol stilbenoid inhibitor, piceatannol, which binds to a pocket formed by contributions from α and β stator subunits and the carboxyl terminal region of the rotor γ subunit. The inhibitor did not interfere with the movement through the 120° rotation step, but caused increased duration of the catalytic dwell. Because all of the beads rotate at a lower rate in the presence of saturating piceatannol and the dissociation rate of the inhibitor is relatively slow, it appears that inhibitor stays bound throughout the rotational catalytic mechanism. Furthermore, piceatannol does not cause a bias in the behavior of the three catalytic dwell positions suggesting that the inhibitor rotates with the γ subunit against the α/β subunits. Arrhenius analysis of the duration of the catalytic dwell shows significantly increased activation energy of the rate-limiting step that triggers the 120° rotation. The activation energy was further increased by combination of piceatannol and the γM23K mutation indicating that the inhibitor and the β-γ interface mutation affect the same transition step, even though they perturb physically separated rotor-stator interactions. Our data indicate that both rotor-stator interaction sites contribute to formation of the rate limiting transition state. These studies were supported by a grant from Ministry of Science and Culture of Japan.

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Characterization of TF_oF_1 ATP Synthase C Subunit Ring in Membranes with HS-AFM and Solid-State NMR

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F_oF_1-ATP synthase is located in the membranes and involved in ATP synthesis coupled with proton-translocation across the membrane. F_1 carries out the rotational catalysis for the ATP synthesis and F_o is responsible for the H^+ translocation coupled with the rotation of *c* subunit ring, which also rotates the gamma subunit of F_1. We have established a new purification method of the *c* subunit rings from the F_oF_1 of *thermophilic Bacillus* PS3 (TF_oF_1) expressed in *E. coli*. They were reconstituted into membranes of dimyristoyl phosphatidylcholine with perdeuterated acyl chains. To examine the macroscopic architecture of the oligomeric TF_oc in the DMPC membranes, successive high speed atomic force microscopy (HS-AFM) images of the reconstituted preparation on the mica in the presence of buffer were obtained at imaging rate of 2-5 frames per second at room temperature. The HS-AFM images of the TF_oc oligomers expressed in *E. coli* and reconstituted into DMPC membranes revealed torus-shaped substances with the pores at the center, confirming that the intact *c*-ring structure is maintained in the purified smple. The similar images were obtained for the oligomeric TF_oc synthesized